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13. ABSTRACT *(Maximum 200 words)*

Seven adults, 2 women in the follicular phase or the menstrual cycle and 5 men, were studied. We intend to provide information relating to age, gender, and the phase of the menstrual cycle when the study is complete. The question addressed to date, regards the mechanism explaining the diurnal urinary response to a water load of 12 ml/kg lean body mass. We observed a 20% greater ($P < 0.02$) diuresis in response to a water load during the daytime compared to nighttime, despite a slightly lower resting plasma osmolality (Posm) during the nighttime. This response appears to be due to a greater reduction ($P = 0.03$) in plasma osmolality of about 2 mOsm during the daytime compared to a slight 1 mOsmkg reduction at nighttime (Not significant). This resulted in a decrease in plasma vasopressin levels that were lower after the drink during the daytime compared to the nighttime ($P = 0.008$). Free water loss accounted for most of the diuretic response as expected, and although at this time the differences are not statistically significant, the increased cumulative urine flow appears to result from a prolonged decrease in urine osmolality and increase in free water clearance during the daytime compared to the nighttime.

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J. R. Clay 11 Sept. 1995

PI - Signature Date

TABLE OF CONTENTS:

INTRODUCTION:

The recent observation (1) that there is an impaired ability to excrete a water load at night in adult men is the first demonstration of a diurnal pattern in water handling *per se* . It has been previously shown that the diuresis and natriuresis associated with either head-out immersion in water or in response to infusion of 2 liters of normal saline is reduced at night (2), but the difference between daytime and nighttime responses was almost entirely due to differences in osmotic (as opposed to free water) clearance (2,3). The mechanisms that lead to the difference in water handling have not been determined. In those original experiments, the major differences between the daytime and nighttime urine output and osmolalities became noticeable 90 minutes after the challenge, and were definitely evident by 2 hours. Measurements of plasma osmolality and ADH were taken once following the challenge at 60 min, and therefore could not provide any information on the potential role of the ADH system in the decreased nocturnal diuretic response. In order to define a potential role of ADH in the diurnal response difference to water loading, measurements of plasma osmolality and ADH must be obtained between 60 and 180 minutes following the drink. There are two probable ADH and osmolality responses to such a water challenge: (1) Either plasma osmolality and ADH will remain lower during the daytime, thereby causing the extended period of decreased urine osmolality and increased urine flow, or (2) the plasma ADH and osmolality will increase similarly during daytime and nighttime, but there would be a comparative refractoriness of the kidney to ADH during the daytime.

The circadian pattern of urine flow, high during the day and low during the night, has been thought to be due to changes in plasma ADH, quoted to be low during the day and high during the night. Although this pattern of ADH has been reported (5), the exceptions to this report are numerous. Most current observations report ADH patterns to be high during the daytime and low during the night in ambulatory subjects(6,7,8,9), but these reports are often ignored (10).

In addition to ADH, cortisol has a definite role in controlling the handling of free water by the body. Cortisol greatly enhances the ability to excrete a water load (4). Serum cortisol levels exhibit a circadian rhythm, being highest at 0800 H and lowest at midnight. This rhythm has been reported to normally begin at about 6 months of age (12), but basal cortisol levels appear to progressively increase and reach adult levels around puberty (13). It is important to recognize that the circadian rhythm of cortisol favors water retention at night and mirrors the circadian rhythm of urine output. The importance of this is suggested by studies in subjects who are given a steady dose of cortisone lose the circadian rhythm of urinary water and sodium excretion (11).

Although a number of factors have been proposed as causing enuresis, a study by Norgaard, et al (10) found that 9 of 11 patients studied had normal bladder capacities, but that 7 of the 11 did not have lower urine flows at night. Therefore, enuretic children represent a population where the adult circadian rhythm of reduced urine flow at night is not completely developed.

The present experiment will indirectly test the hypothesis that cortisol may play a role in the adult circadian rhythm of reduced urine flow at night. We hypothesize that in the pediatric enuretic subjects, the normal adult cortisol rhythm will not be present, but will be found in non-enuretic children.

The response of adult women to a water load has not been compared to men. The relationship of ADH to plasma osmolality has been studied in various conditions in female rats that were pregnant or pseudopregnant, or infused with estrogen and progesterone combinations, and was found to be unchanged (14). However, the effectiveness of ADH in causing an antidiuresis is greatly influenced by the presence of estrogen or progesterone. Share and Crofton (15) recently reviewed this topic, and it appears that estrogen and progesterone have separate, additive effects in inhibiting the effects of ADH on the kidney. This appears to be through an inhibition of ADH-induced production of cyclic AMP(16). This clearly implicates that estrogen and progesterone have an effect on water balance which is distinct from other effects which they have on sodium balance (estrogen causes sodium retention, while progesterone has a minimal sodium loosing effect). These observations suggest that if a standardized oral water load is administered acutely to men and women, the body distribution and reduction in plasma osmolality should be similar. The subsequent reduction in plasma ADH should also be similar, but the proportional volume of water excreted should be greater in women due to the antagonism which estrogen and progesterone exert on ADH. Since this effect appears to be dependent upon estrogen and progesterone levels, the effect should be greatest in adult females during the luteal phase. In view of the similarity of sex hormone levels prior to puberty, the difference between males and females would not be expected in the children.

EXPERIMENTAL METHODS:

The schedule is as shown below. The daytime and nighttime experiments will be separated by more than 24 hours. Breakfast and supper will have been eaten at about 0600 and 1800, with instructions to limit drinks to approximately one cup of fluid, and similar caloric and fat contents in the meals, and no alcohol or smoking on the day of the experiment. Because of the late hour of the nighttime test, 1900 until 2300, we will allow the children to spend the night at TAMC if so desired, without charge. Also, the children will have only hourly urine collections.

Table 1: Schedule

Daytime	Nighttime	Activity or Event
0600	1800	Void urinary bladder (this request will be relayed to the subjects telephonically)
0700	1900	Subjects report to study area. Body weights and skin folds are determined. Urine #1 is collected. Adult subjects are prepared with a 21 ga. "Angiocath" intravenous line with heparinized (1:1 heparin [1000 units/ ml] to normal saline) saline in the line.
0800	2000	Subjects void bladders. Urine collection #2. Record blood pressure and heart rate and temperature. Obtain blood sample #1 (20 ml for adults, or optional 10 ml for children)

(Table 1 continued)

Daytime	Nighttime	Activity or Event
0800 - 0815	2000 - 2015	Drink water, 15° C, 12 ml/kg lean body weight
0830	2030	Collect urine sample #3, & blood sample #2 (12 ml each for remainder of test)
0900	2100	Collect urine sample #4 & blood sample #3
0930	2130	Collect urine sample #5 & blood sample #4
1000	2200	Collect urine sample #6 & blood sample #5
1030	2230	Collect urine sample #7 & blood sample #6
1100	2300	Collect urine sample #8 & blood sample #7

Evaluations to be made on specimens.

Blood. (only in adult studies)
- Hematocrit (microcentrifugation)

Plasma (only in adult studies, and for initial samples in consenting children)
- Estrogen (adult females only, only on initial sample)
- Progesterone (adult females only, only on initial sample)
- Aldosterone (only on initial sample)
- Dehydroepiandrosterone (only on initial sample)
- Cortisol
- ACTH (initial samples only)
- Plasma Renin Activity (initial samples only)
- Antidiuretic hormone
- Epinephrine
- Norepinephrine
- Osmolality (Freezing point depression)
- Sodium (ion specific electrode)
- Potassium (ion specific electrode)
- Creatinine (Jaffe reaction , auto analyzer)

Urine (all subjects)
- Volume
- Creatinine
- Osmolality
- Sodium
- Potassium
- Cortisol
- Antidiuretic hormone
- Aldosterone (initial sample only)
- DHEA (initial sample only)
- Epinephrine
- Norepinephrine

Calculated values in adults include, creatinine clearance as an index of glomerular filtration rate, osmotic and free water clearances, and filtration fraction of sodium and potassium throughout the experiment.

RESULTS:

No pediatric studies have been conducted, and since so few female subjects have been completed, we can make no statements about pediatric, gender or menstrual cycle effects on the responses to a water load at this time. We have conducted studies on two females in the follicular phase of the menstrual cycle, and none in the luteal phase, and we have conducted studies on 5 men. Not all of the analyses on these studies is complete.

So far, the data are consistent with the previously published observation that the cumulative urine volume produced in response to a drink of water is greater during the daytime than the nighttime (Figure 1).. Overall, the cummulative urine flow during the daytime is greater (using orthoganal comparisons), P=0.008

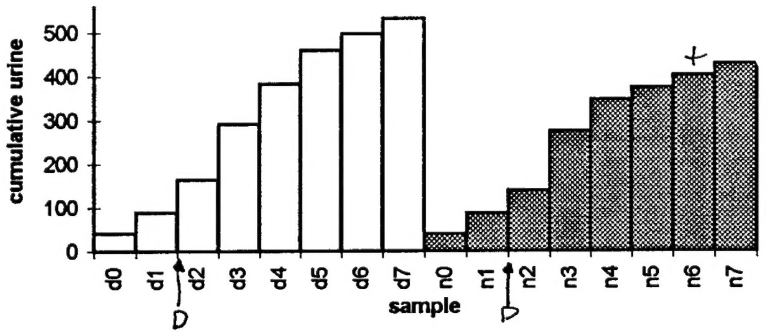

Figure 1. Cumulative urine flow shown in 30-min increments in 5 male subjects and 2 female subjects in the follicular phase of the menstrual cycle, in response to a water load of 12 ml/kg lean body mass, at daytime (samples d0 - d7, open bars)and nighttime (samples n0 -n7, shaded bars). + = $P < 0.05$ compared to corresponding value during the daytime. D = point at which water drink was given.

The increased urine flow during the daytime is not due to a state of greater hydration. By controlling the food and fluid intake before both daytime and nighttime experiments, the plasma osmolality was not greater at night. (Figure 2). In fact, the average plasma osmolality was slightly lower (not statistically significant) prior to the water load when given during the nighttime. It is noteworthy that plasma osmolality is decreased during the daytime (P=0.03) when all post drink values are compared to the predrink control but this was not statistically significant during the nighttime. Geelen, et al (17), when documenting an oropharyngeal inhibition of vasopressin release in humans, showed that plasma osmolality is reduced about 1 mOsm/kg at 30 min and 60 min post drink (10 ml/kg). The drop we observed during the daytime is slightly greater, but is consistent with their observation. The difference in the plasma osmolality response between nighttime and daytime may, indeed be an important finding, and could account for the difference in urine flow that occurs.

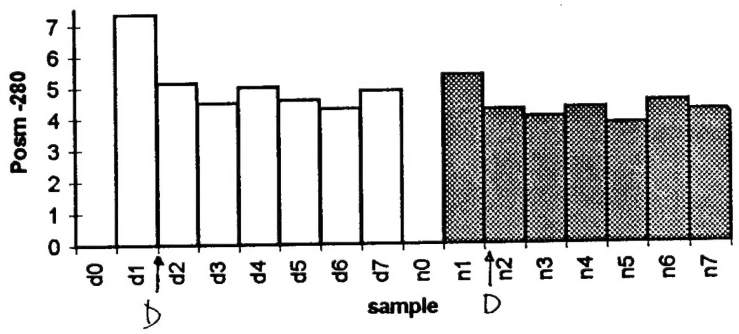

Figure 2. Plasma Osmolality is shown on the y-axis less 280 (mOsm/kg H_2O). No plasma samples were taken at sample periods d0 and n0.

If the mechanism of this increased urinary response during the daytime is due to the decrease in plasma osmolality, we would postulate that its effect on urine flow would probably be via reduced vasopressin, and a subsequent greater reduction in urine osmolality. This should result in a greater free water excretion during the daytime. Figure 3 shows the importance in free water clearance (CH_2O) in the diuretic response. Note that, CH_2O increases about 2.5 ml/min and the increase, remaining at about 1 ml/min, is extended to 1.5 h. By comparison, the increase in Cosm is only 1 ml/min and lasts only 30 min. It also appears that the increase in CH_2O lasts longer during the daytime. In fact, the integrated response of increased free water loss is greater during the daytime.

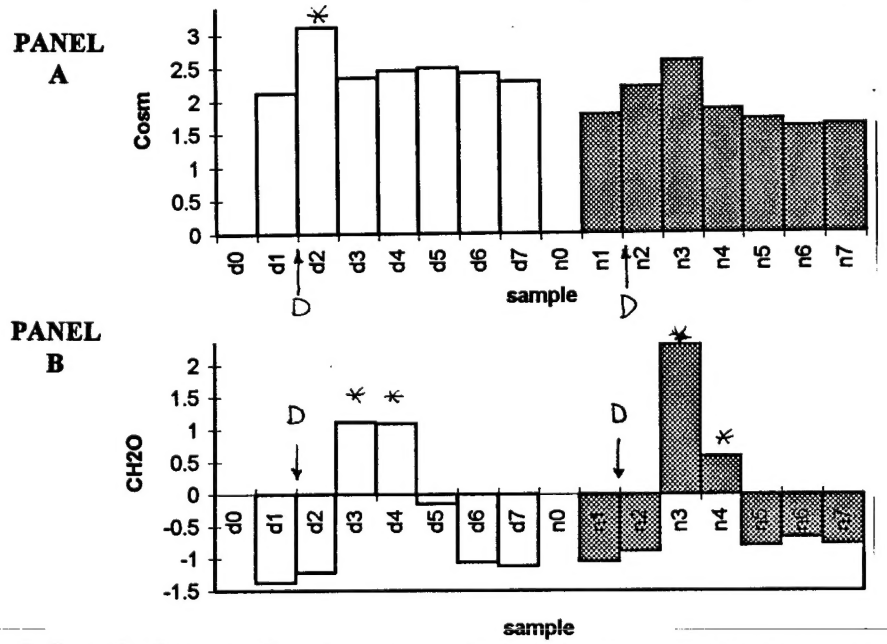

Figure 3. Osmotic clearance (Cosm), panel a, and free water clearance (CH_2O), panel b. Note the increase in CH_2O following the drink accounts for a ajority of the diuresis. Also, the response tends to last longer in the daytime experiments.

The urine osmolality (Uosm), shows a typical response with a tendency for a more prolonged reduction in osmolality during the daytime than the nighttime (Figure 4). This eefct is not statistically significant at this time, but is identical to our previous findings (1).

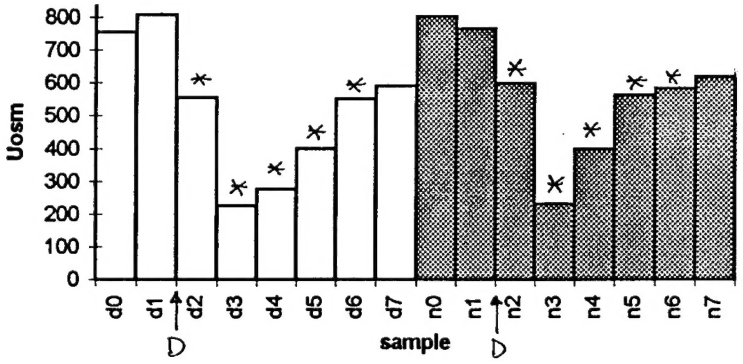

Figure 4. Urine osmolality (Uosm, mOsm/kg H_2O). Note the more prolonged decrease during the daytime.

The question now becomes, is this due to decrease in vasopressin? This has never been previously determined, and the current studies indicate that the mechanism may be vasopressin related. Figure 5 shows the plasma vasopressin (Pavp) and urinary vasopressin (Uavp) in response to the drink of water. The plasma vasopressin shows a greater and more prolonged reduction during the daytime and this is generally confirmed by the urinary excretion of vasopressin.

CONCLUSIONS:

At this point our data support previous observations, and suggest a vasopressin-mediated difference in the day/night response to a water load. That is, the water drink during the daytime causes a more prolonged reduction in plasma osmolality and consequently a more prolonged decrease in plasma vasopressin concentration than during the nighttime. This would explain the previously observed and presently confirmed (albeit not statistically significant at this point)observation that urine osmolality is decreased for a more prolonged time during the daytime than the nighttime in response to an identical water load.

These observations dictate that a mechanism would have to exist that causes plasma osmolality to stay down longer in response to a water load during the daytime than the nighttime? Since exogenous cortisol can delay the increase in plasma osmolality resulting from exogenous administration of hypertonic saline in human subjects (18), it may be that the circadian rhythm of cortisol, high during the morning, would delay the movement of water out of the extracellular space in a manner similar to the experiments of Aubry et al (18). The current experiments will not specifically address this hypothesis, but since vasopressin seems to be involved in the day/night response, we may find some differences due to gender and age in subsequent experiments on this protocol, and supported by this grant.

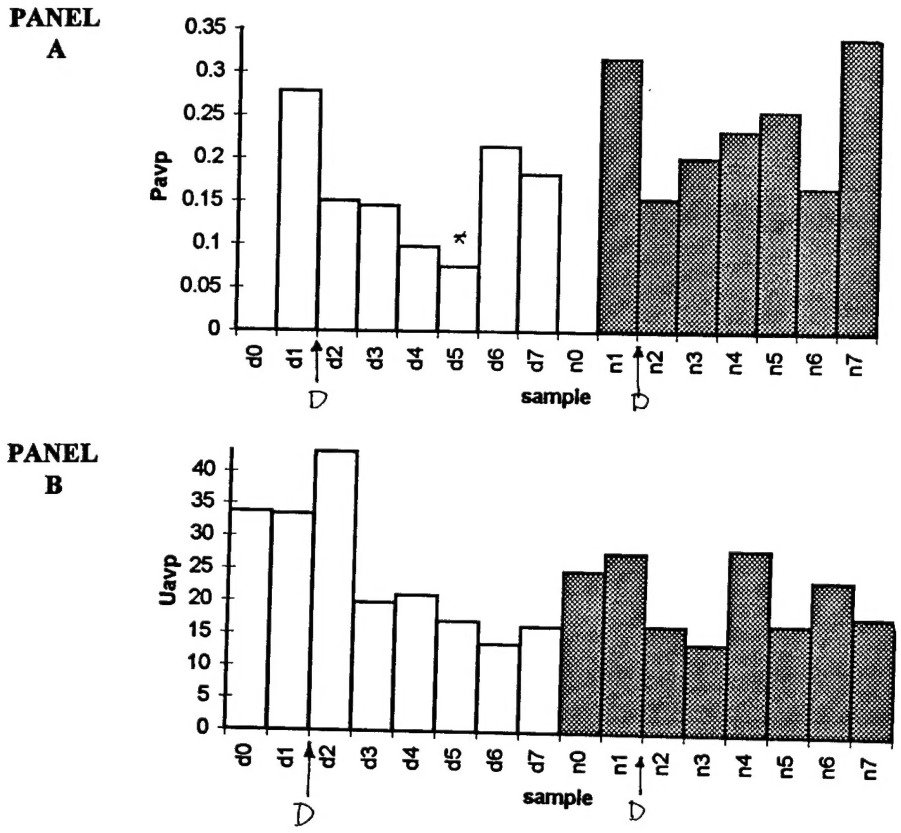

Figure 5. Plasma vasopressin concentration (Pavp, μU/ml), panel a, n = 5, and urinary vasopressin (Uavp, μU/mg creatinine), panel b, n = 4. Note the more prolonged decrease in Pavp during the daytime. This pattern is qualitatively reflected by the Uavp.

We have had delays in initiating this project, and some initial difficulties in achieving compliance to the rigid food and water intake requirements necessary before the waterload experiment, but we feel confident that the studies will be completed this fall.

REFERENCES

1. Takeuchi, H, Mohri, M., Shiraki, K., Lin, Y.C., Claybaugh, J.R., and Hong, S.K. Diurnal renal responses to water loading at sea level and 31 atm abs. Undersea Hyperbaric Med. 22:61-71, 1995.

2. Krishna, G.G., and Danovitch, G.M., Renal responses to central volume expansion in humans is attenuated at night. Am. J. Physiol. 244(Regulatory Integrative Comp. Physiol. 13): R481-R486, 1983.

3. Shiraki, K., Konda, N., Sagawa, S., Claybaugh, J.R., and Hong, S.K. Cardio-renal-endocrine responses to head-out immersion at night. J. Appl. Physiol. 60:176-183, 1986.

4. Cornette-Finn, K. ADH response to peripheral and central cortisol administration. Ph.D. Thesis, University of Hawaii, 1987.

5. George, P.L.C., Messerli, F.H., Genest, J., Nowaczynski, W., Boucher, R., Kuchel, O., and Roja-Ortega, M. Diurnal variation of plasma vasopressin in man. J. Clin. Endocrinol. Metab. 41:332-338, 1975.

6. Claybaugh, J.R., Hong, S.K., Matsui, N., Nakayama, H., Park, Y.S., and Matsuda, M. Responses of salt- and water-regulating hormones during a saturation dive to 31 ATA (Seadragon IV). Undersea Biomed. Res. 11:65-80, 1984.

7. Claybaugh, J.R., Matsui, N., Hong, S.K., Park, Y.S., Nakayama, H., and Shiraki, K. Seadragon VI: A 7-day saturation dive at 31 ATA. III. Alterations in basal and circadian endocrinology. Undersea Biomed. Res. 14:401-411, 1987.

8. Claybaugh, J.R., Goldinger, J.M., Moon, R.E., Fawcett, T.A., Exposito, A.G., Hong, S.K., Holthaus, J., and Bennett, P.B. Urinary vasopressin and aldosterone and plasma volume during a dry saturation dive to 450 m. Undersea Biomed. Res. 19:295-304, 1992.

9. Luboshitzky, R., Lavie, P., Sok, Y., Glick, S.M., Leroith, D., Shen-Orr, Z., and Barzilai, D. Antidiuretic hormone secretion and urine flow in aged catheterized patients. J. Life Sciences, 8:99-103, 1978

10. Norgaard, J.P., Pedersen, E.B., and Djurhuus, J.C. Diurnal anti-diuretic hormone levels in enuretics. J. Urol. 134:1029-1031, 1985.

11. Rosenbaum, J.D., Ferguson, B.C., Davis, R.K., and Rossmeisl, E.C., The influence of cortisone upon the diurnal rhythm of renal excretory function. J. Clin. Invest. 31:507-520, 1952.

12. Onishi, S. Mijazawa, G, Wishimura, Y., Sugiyama, S., Yamakawa, T., Inagaki, H., Katoh, T., Itoh, S. and Isobe, K., Postnatal development of circadian rhythm in serum cortisol levels in children. Pediatrics 72:399-404,1983.

13. Winter, J. Physiology and pathology of adrenocortical function in infancy and childhood. In: *Pediatric Endocrinology, 2nd edition.* Collu, R. , Ducharme, J.R., and Guyda, H.J. (Eds.)., Raven Press, New York, pp473-507, 1989.

14. Lindheimer, M.D., Barron, W.M., and Davidson, J.M., Water metabolism and vasopressin secretion in pregnancy. In: *Vasopressin*, Schrier, R.W. (Ed). pp229-240, 1985.

15. Share, L. and Crofton, J.T., Interactions between the gonadal steroid hormones and vasopressin and oxytocin. In: *The Neurohypophysis: A Window on the Brain Function,* North, W, Share, L. and Moses, A (Eds), pp 438-454, 1993.

16. Hatano, T., Ogawa, K., Kanda, K., Seo, H., and Matsui, N.. Effect of ovarian steroids on cyclic adenosine 3':5'-monophosphate production stimulated by arginine vasopressin in rat renal monolayer cultured cells. Endocrinol. Jpn. 35: 267-274, 1988.

17. Geelen, C, Keil, L.C., Kravik, S.E., Wade, C.E., Thrasher, T.N., Barnes, P.R., Pyka, G., Nesvig, C., and Greenleaf, J.E., Inhibition of plasma vasopressin after drinking in dehydrated humans. Am. J. Physiol. 247 (Regulatory Integrative Comp Physiol 16): R968-R971, 1984.

18. Aubry, R.H., H.R. Nankin, A.R. Moses, and D.H.P. Streeten. Measurement of the osmotic threshold for vasopressin release in human subjects, and its modification by cortisol. J. Clin. Endocrinol. Metab. 25:1481-1492, 1965.